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Evaluation of an Accelerated Protocol for Detection of Extended-Spectrum β-Lactamase-Producing Gram-Negative Bacilli from Positive Blood Cultures

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We evaluated a protocol for the accelerated detection of extended-spectrum β-lactamases (ESBLs) in gram-negative bloodstream pathogens. Two hundred eighty-three blood culture bottles were subjected to direct ESBL testing by inoculating samples directly from blood culture bottles onto agar plates containing cefotaxime and ceftazidime disks, with and without clavulanate. Standard ESBL testing in accordance with the NCCLS guidelines after subculturing on agar plates was performed in parallel. Results of the direct ESBL testing were reported 2.3 days sooner and were comparable to those of the standard NCCLS method with sensitivity, specificity, and positive and negative predictive values of 100, 98, 94, and 100%, respectively.

Production of extended-spectrum β-lactamases (ESBLs) is the most prevalent mechanism of resistance to broad-spectrum cephalosporins among species of *Enterobacteriaceae* that do not carry chromosomal β-lactamases (4). The proportion of ESBL producers among hospital isolates varies between geographical areas (12). In the United States, ESBLs are produced by more than 25% of intensive care unit *Klebsiella pneumoniae* isolates (10, 12), and in some parts of Europe, Asia, and South America they are much more common (1). The spread of this mechanism of resistance is therefore an emerging problem. In Israel, ESBLs are common among members of the family *Enterobacteriaceae*. In our hospital, we found an ESBL-producing phenotype in 20% of clinical isolates causing gram-negative infections (8). Failure to detect and report ESBL production by gram-negative bacteria in a timely manner may result in a significant delay in appropriate antimicrobial treatment. Such delays have been shown to result in increased mortality, morbidity, and health care expenditure (3, 5, 6).

To reduce the burden on the microbiology laboratory, the NCCLS recommends that a screening process be applied and that only organisms for which the MICs of broad-spectrum cephalosporins or aztreonam are increased be tested for the ESBL phenotype (7). This procedure delays reporting of ESBL-producing organisms by 3 to 4 days and is therefore inappropriate for institutions such as ours, where ESBLs are highly prevalent.

In an earlier study, we examined the feasibility of detection of ESBL producers directly from blood culture bottles by experimentally inoculating blood culture bottles with defined strains of *Escherichia coli* and *K. pneumoniae* and volunteer blood and found this method to produce results similar to those of the method recommended by the NCCLS (9). The objectives of the present study were to evaluate the performance of this direct ESBL testing method with clinical gram-negative bloodstream isolates received in our microbiology laboratory and to assess the impact on reporting time.

Blood cultures submitted to our clinical microbiology laboratory between June 2003 and December 2003 were examined. Repeat samples were excluded. Bottles were incubated in the BacT/ALERT system (bioMérieux Inc., Durham, N.C.). Only bottles demonstrating growth of a single gram-negative bacillus were included. The final identification of each blood isolate tested for ESBL production was determined by the VITEK-2 system (bioMérieux, Marcy l'Étoile, France) used with the ID-GNB card for identification of gram-negative bacilli.

The ESBL phenotype (both in the direct test and in the standard test) was determined by the NCCLS-recommended ESBL confirmatory disk diffusion assay by the Oxoid combination disk method (2). Two cephalosporin-containing disks (30 μg; Oxoid, Basingstoke, Hampshire, England) containing cefotaxime and ceftazidime with and without 10 μg of clavulanic acid were used. Results were interpreted as positive for ESBL if the zone of inhibition of the combination disk was more than 5 mm greater than that of its corresponding cephalosporin disk without clavulanic acid, in accordance with NCCLS guidelines (7).

For accelerated ESBL testing, upon a growth signal from the BacT/ALERT system, 0.2 ml of liquid from every blood culture bottle exhibiting gram-negative growth was withdrawn aseptically with a syringe, swab spread onto Mueller-Hinton agar plates (HyLabs, Rehovot, Israel), and tested for ESBL produc-

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TABLE 1. Comparison of direct and standard tests for ESBL detection in gram-negative isolates recovered from blood cultures

Microorganism(s)	No. (%) of isolates	No. (%) of ESBL-positive isolates	
		Direct test	Standard test
Escherichia coli	97 (34.3)	11 (11.3)	11 (11.3)
Klebsiella spp.	59 (20.8)	26 (44)	26 (44)
Pseudomonas aeruginosa	29 (10.2)	1 (3.4)	1 (3.4)
Acinetobacter baumanni	25 (8.8)	2 (8)	1 (4)
Enterobacter spp.	17 (6)	5 (29.4)	4 (23.5)
Proteus spp.	17 (6)	4 (23.5)	4 (23.5)
Serratia marscens	9 (3.2)	0	0
Providencia stuartii	8 (2.8)	5 (62.5)	5 (62.5)
Citrobacter spp.	6 (2.1)	1 (16.7)	0
Salmonella spp.	4 (1.4)	0	0
Morganella morganii	2 (0.7)	1 (50)	1 (50)
Stenotrophomonas maltophilia	2 (0.7)	1 (50)	1 (50)
Other gram-negative bacteria (not fully identified)	8 (2.8)	0	0
Total	283	57 (20.1)	54

tion as described previously (9). *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 served as negative and positive controls for ESBL production, respectively.

Standard ESBL testing was performed in parallel with the accelerated ESBL test. The contents of each bottle tested were subcultured on agar plates, and the pure culture was identified to the species level with the VITEK-2 system. The pure culture obtained from each bottle tested was tested for the ESBL phenotype with a suspension of a 0.5 McFarland turbidity standard prepared in brain heart infusion broth (HyLabs), followed by the disk diffusion assay as detailed above. The sensitivity, specificity, and positive and negative predictive values of the accelerated test were calculated by using the standard method as the reference. Concordance of the two tests was calculated with McNemar's exact test, and time to reporting was examined with the Wilcoxon signed-ranked test for matched observations.

A total of 283 blood culture bottles grew a single aerobic gram-negative bacillus and were subjected to direct ESBL testing. Two hundred fifteen isolates (76%) belonged to the family *Enterobacteriaceae*, and 54 (19%) were identified as nonfermenters. The most frequently isolated species were *E. coli* and *K. pneumoniae* (35 and 21%, respectively), followed by *Pseudomonas aeruginosa* (10%) and *Acinetobacter baumannii* (9%) (Table 1).

When tested directly from blood culture bottles, 57 (20%) of these 283 blood isolates had an ESBL phenotype, while this phenotype was found in 54 (19%) by the standard test. The sensitivity, specificity, and positive and negative predictive values of the accelerated ESBL test were 100, 98, 94, and 100%, respectively. The ESBL-producing phenotype was found most frequently among *Klebsiella* isolates (45%, all *K. pneumoniae*), with a concordance of 100% between the direct and standard ESBL tests. The second most prevalent ESBL producer was *E. coli*, for which there was likewise a concordance of 100% between the two testing methods.

When the accelerated ESBL protocol is applied to gram-negative bacilli, it is also applied to organisms for which no standard recommendations for ESBL testing exist (11). For this study, we applied the NCCLS recommendations for *E. coli* and *K. pneumoniae* to all gram-negative organisms. We have previously reported an excellent correlation between the phenotypic assays and the presence of ESBL-encoding genes in non-*E. coli* and non-*Klebsiella* isolates of *Enterobacteriaceae* in Israel (M. M. Morlote, S. Navon-Venezia, Y. Carmeli, L. Venkataraman, and H. S. Gold, 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-55, 2003; J. Schlesinger, S. Navon-Venezia, I. Chlemintzki, O. Hammer-Munz, M. Schwaber, and Y. Carmeli, Abstr. Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. 1866, 2004). In the present study, 16 of 53 ESBL-producing isolates were non-*E. coli* and non-*Klebsiella* species of *Enterobacteriaceae*. Among nonfermenters, the ESBL-producing phenotype was uncommon, being found in only 4 of 56 isolates. The correlation between a positive result in this phenotypic assay and the presence of ESBL-encoding genes in nonfermenters is not fully known.

The mean time from Gram staining to detection of ESBL production by conventional testing was 3.38 ± 0.49 days versus 1.05 ± 0.23 days by direct testing. Thus, the average time difference between the two tests was 2.3 ± 0.47 days ($P < 0.0001$).

Of 57 isolates identified as ESBL producers by the direct test, 3 were not identified as such by the standard method (1 of *A. baumannii*, 1 of *Enterobacter cloacae*, and 1 of *Citrobacter braakii*). This discrepancy may represent either false-positive results obtained by direct testing or false-negative results obtained by the standard test, perhaps as a result of plasmid loss by the bacteria during longer processing of cultures. From a therapeutic standpoint, the discrepancy in these cases is of little relevance as two of the three isolates (*A. baumannii* and *E. cloacae*) were resistant to all broad-spectrum cephalosporins.

In conclusion, we examined the performance of an accelerated protocol for detection of ESBLs directly from blood cultures containing gram-negative bacilli and demonstrated that the results of the direct ESBL test are highly concordant with the results of the standard test. This test allows reporting of ESBL production an average of 2.3 days sooner than via the conventional test. Since the accelerated protocol exhibited 100% sensitivity relative to the standard protocol, availability of the results of direct testing to clinicians would result in earlier treatment of bacteremia by all ESBL-producing organisms that would ultimately be identified by the standard protocol.

We believe that the results of this work provide enough evidence for the use of the accelerated protocol, in addition to standard methods, by clinical microbiology laboratories in areas with a high prevalence of ESBLs. Indeed, in light of our results, our clinical microbiology laboratory has added direct ESBL testing from blood culture bottles to its standard methods of ESBL detection. Further studies are required to provide additional clinical validation of our findings and thereby to determine whether the accelerated protocol is reliable enough to replace standard methods of ESBL detection in patients with gram-negative bacteremia.

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